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Timing Is Everything

Changes in sensory experience are capable of producing changes in sensory transmission within the neocortex. For example, closing one eye of a binocular animal during the critical period leads to depression of cortical responses to stimulation of the deprived eye. This can produce an inability to see through the deprived eye once the closed eye is reopened and a loss of stereoscopic vision (Wiesel, 1982). Similarly, deprivation of a number of whiskers on the face of a rodent leads to depression of cortical responses to the deprived whisker after the whiskers have regrown. These results raise the question of how sensory information (or a lack of it) can produce depression of responses within the cortex. Two general possibilities exist: either inactivity at a particular synapse leads to passive decay of synaptic strength, or some form of neuronal activity is required to weaken the deprived inputs. If activity is required, then either the spared inputs are involved in driving down the synaptic gain of the deprived inputs (heterosynaptic depression), or the pattern of activity at the deprived synapses is responsible for weakening their own synaptic gain (homosynaptic depression).

A mechanism that captures elements of both hetero- and homosynaptic depression is the covariance or extended Hebb rule (see Fox et al., 1998). Here, the relationship between pre- and postsynaptic activity determines whether the synapse weakens or strengthens. If pre- and postsynaptic activity occur at the same time the synapse strengthens, whereas if they occur at different times the synapse weakens. Because postsynaptic activity can be controlled by spared inputs, active inputs do have a heterosynaptic influence on the quieter deprived inputs. On the other hand, presynaptic activity that is insufficient to drive the postsynaptic cell will also lead to homosynaptic depression.

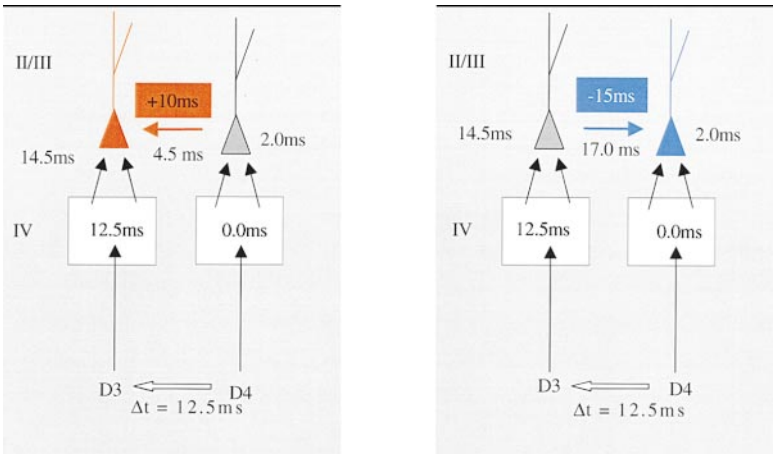
A physical manifestation of the covariance rule has been described for a number of CNS synapses. Earlier papers showed the importance of the relationship between pre- and postsynaptic activity in determining the direction of synaptic gain change in the hippocampus (Kelso et al., 1986; Stanton and Sejnowski, 1989). Recently, studies at other synapses have also explored the role of timing differences in synaptic plasticity and have shown that the time window for coincidence of pre- and postsynaptic activity is relatively brief (Markram et al., 1997; Zhang et al., 1998). LTP is found with positive delays of +20 ms and LTD is found with negative delays of about −20 ms.

In a paper in this issue of *Neuron*, Feldman uses the well-defined architecture of the barrel cortex to examine synaptic depression mechanisms in a pathway between layer IV and layer III neurons within the same cortical column. The barrel cortex is part of the rodent somatosensory cortex and receives information from the whiskers (Van der Loos and Woolsey, 1970). The layer IV to III synapse is of particular interest, as it has been implicated in depression of sensory responses in superficial layers of the cortex following whisker deprivation (Glazewski and Fox, 1996). To test whether the relative timing of pre- and postsynaptic activity has an effect on cortical plasticity, Feldman prepared slices of barrel cortex and made whole-cell recordings from layer III neurons. The timing of presynaptic action potentials was controlled by stimulating layer IV cells extracellularly, while the timing of postsynaptic potentials was controlled by positive current pulses applied to the postsynaptic neuron.

The exact timing of pre- and postsynaptic action potentials in the layer IV to layer III pathway was found to be critical for induction of plasticity. No change occurred if the two events were grossly out of alignment, so, for example, a 500 ms delay between pre- and postsynaptic activity did not alter the characteristics of the synapse. However, if presynaptic activity occurred before the postsynaptic activity (a positive delay) by between 0 and 20 ms, LTP was observed. Conversely, if a postsynaptic action potential occurred before the presynaptic depolarization (a negative delay) by between −100 and 0 ms, then LTD occurred (Feldman, 2000).

What Feldman found that was slightly unusual in the layer IV to III pathway is that the time window for LTD is far longer than that for LTP. This simple fact has several implications for models of timing-based plasticity at these synapses. For example, this plasticity mechanism enables the neuron to form a naturally stable set of synapses without all its inputs being in danger of saturating. This is because of the negative feedback relationship between a change in synaptic gain and the output of the neuron. If a number of synapses show correlated activity and are therefore able to drive the cell more powerfully, more postsynaptic spikes are produced. Subsequently, the general increase in postsynaptic spikes will mean that many spikes form chance coincidences with presynaptic activity. Since these chance events are more likely to occur in the longer time window for LTD than in the time window for LTP, the set of synaptic weights for the cell's uncorrelated inputs will automatically decrease. In this way the total set of synaptic weights tend to stability.

A second consequence of the plasticity rule observed by Feldman is that it would tend to favor the development of connections that activate layer III cells within a narrow time window. There is evidence for this in adult animals. When the whiskers activate the barrel cortex, layer IV cells produce a relatively rapid and synchronous discharge, typically starting within about 10 ms of one another. Neurons in layers II and III, which receive columnar input from the layer IV cells, respond after the layer IV cells in their column (96% in layer II and 84% in layer III): furthermore, they start to respond within 5–6 ms of their counterparts in layer IV (Armstrong-James et al., 1992). The spread of timing differences between layers IV and II/III is therefore about +16 ms. Whisker stimulation would therefore create natural timing differences that fall within the LTP time window for columnar



The Sequence of Activation within Barrel Cortex following Stimulation of Two Whiskers (D3 and D4) 12.5 ms Apart

All times are relative to activation of the earliest cell to respond in layer IV. The panel on the left shows activation of the layer IV barrel for D4 at 0 ms. Approximately 2 ms later, cells in layer II/III of the same column are activated by convergence of input from layer IV cells. If this cell is brought to threshold and fires an action potential, these synapses would potentiate according to the results described by Feldman (2000). The neighboring barrel is activated at 12.5 ms due to the delay between activaton of the two whiskers. Approximately 2 ms later than this, at 14.5 ms total delay, layer IV cells activate layer III neurons in the same D3 column. The columnar input arrives earlier than that time from the neighboring barrel to converge on the same neuron at about +4.5 ms and for this illustration is subthreshold for firing the cell. The net timing difference between the two whisker inputs is therefore +10.0 ms, which is the optimum interval for producing potentiation at the horizontal connection between the barrels. The panel on the right shows the same sequence continuing further in time. The D3 neurons now transmit excitation to the D4 barrel, but because these cells have already been activated, the horizontal input arrives −15 ms after the layer II/III cells have depolarized. This would lead to LTD at the horizontal synapses according to the results described by Feldman.

input. However, inputs from neighboring barrels would be expected to affect this result depending on their relative time of arrival (see figure).

Likewise, the radial transmission of excitation out of layer IV is disrupted by whisker deprivation in a manner that can also be explained by the same plasticity rule. When several whiskers are deprived for a period of time and then allowed to regrow, responses to stimulation of the principal whisker are at normal levels in layer IV, while responses in layers II and III are highly depressed or absent altogether (Fox, 1992). This effect can also be seen in rats and mice as old as 1 month but not in older animals (Glazewski and Fox, 1996; Glazewski et al., 1996). It has been suggested that the reason for the lack of response in superficial layers is a failure of transmission within the layer IV to II/III pathway (Glazewski and Fox, 1996). It had originally been thought that heterosynaptic depression might be involved because the degree of depression increases the closer the cell is located to an active barrel (i.e., a barrel for which the whisker has been spared) (Glazewski and Fox, 1996), and because the depression is greater for the deprived whisker's column of cells the more spared whiskers there are that surround it (Wallace and Fox, 1999).

The asymmetric nature of the plasticity rule governing synaptic changes in layer IV to II/III pathways offers an alternative explanation for these whisker deprivation results. Whisker deprivation may lead to uncorrelated spontaneous activity in the layer IV cells corresponding to the deprived whisker. However, the layer II/III cells would still be expected to depolarize and produce postsynaptic action potentials if neighboring whiskers composing their surround receptive fields are left intact. If the presynaptic activity from the deprived pathway were entirely random, it would fall within the time window for depression more often than that for potentiation because the depression time window is wider (100 ms versus about 20 ms). Hence, the combination of surround receptive field responses and uncorrelated presynaptic spontaneous activity would lead to the depression of responses, which is the experimentally observed end point.

Indeed, the timing rules model proposed by Feldman is compatible with other experimental findings as well. One would predict from this model that the more often the postsynaptic cell fires the greater the depression, and the less it fires the smaller the depression. In fact, there is evidence for both effects. First, if the cells in the deprived whiskers column are surrounded by a "sea" of active columns (produced by sparing all the surrounding whiskers), depression is greater than if the column is surrounded by equally quiet columns expressing spontaneous activity (produced by depriving all the whiskers simultaneously) (Glazewski et al., 1998). Furthermore, if cortical activity is prevented in layers II and III by applying muscimol to the cortex, depression does not occur at all (Wallace, 2000). These results all argue that depression of synaptic strength requires cortical activity and argue against the idea that synaptic strength might decay passively in the absence of activity.

The fact that the previously observed experimental effects are compatible with a timing-based model for plasticity provides evidence that a pre- and postsynaptic timing rule can explain results in vivo. The temporal correlations required seem far more likely to occur in neocortex in reality than the firing patterns used thus far to produce LTP (100 Hz stimulation) and LTD (900 pulses at 1 Hz) in the hippocampus. These results also make the specific prediction that whisker deprivation should result in random spontaneous activity in the deprived barrels' columns. This could be tested with current methods of in vivo recording from behaving rats.

These results also raise questions about what sorts of cellular and molecular mechanisms might mediate these effects at the synapse. Both LTP and LTD induced by spike coincidence are dependent on NMDA receptors (Feldman, 2000). However, NMDA receptors once bound by glutamate are potentially open for 100 ms or more. Why, then, is the time window for LTP just 20 ms? Similarly puzzling is the time window for depression because, in this case, the synapse is able to register the occurrence of a postsynaptic spike for 100 ms pending the arrival of presynaptic activity and hence NMDA receptor activation.

Clearly, the brain has yet to yield up many if not most of its secrets regarding synaptic plasticity, but the ability of a timing-based mechanism to explain system level plasticity with physiologically plausible firing patterns strongly suggests that this is the right track. Time will tell!

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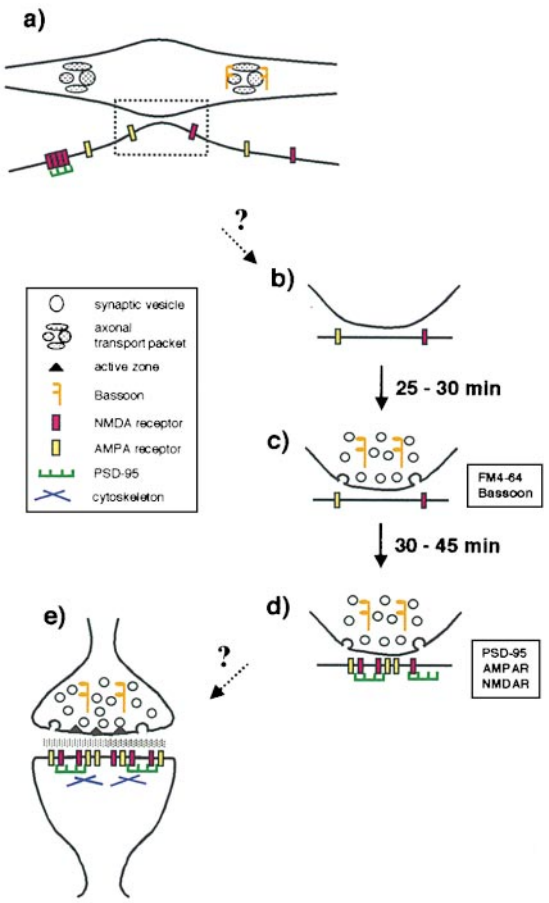

Sequence of Events Leading to Glutamatergic Synapse Formation

(a) Before initial contact, aggregates of synaptic vesicle components and cytomatrix proteins such as Bassoon are transported in the axon (axonal transport packets). In the dendrite the AMPA receptor is diffusely distributed, but NMDA receptor and the scaffolding molecule PSD-95 can form clusters. These preassembled aggregates may contribute to subsequent rapid synaptogenesis.

(b–d) The sequence of events determined in the current study. (b) Axodendritic contact leads to an unknown signaling interaction that results after 25–30 min in differentiation of the presynaptic site (c) seen as FM 4-64 and Bassoon puncta. (d) PSD-95, NMDA receptor, and AMPA receptor cluster at the postsynaptic site after another 30–45 min.

(e) Further maturation may be necessary to form a fully differentiated glutamatergic synapse.

Birth of a Synapse: Not Such Long Labor

Synapses are specialized junctions between cells where the presynaptic cell accumulates synaptic vesicles and release machinery and a postsynaptic cell concentrates neurotransmitter receptors and signaling machinery. Previous studies of synaptogenesis using population analyses of fixed tissue have suggested that it may take days to weeks to form this junction. In this issue of *Neuron*, Ziv and colleagues (Friedman et al., 2000) provide evidence from dynamic analyses of cultured hippocampal neurons that the time required to concentrate presynaptic and postsynaptic molecules at glutamatergic central synapses may be surprisingly short.

The authors combined time lapse imaging of newly formed presynaptic specializations in living neurons with retrospective immunolabeling to follow the differentiation of single synaptic contacts at defined times after formation. Functional presynaptic specializations were identified by stimulation-driven uptake into and release from synaptic vesicles of the amphipathic fluorescent dye FM 4-64. Newly formed FM 4-64 puncta could be assigned a time of birth within a time frame of minutes by repeated loading and unloading of the dye at various time intervals throughout which the cell was imaged. Using this approach, Ziv and colleagues determined